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TITLE:
Potential Side Effect of Inadvertent Intravascular Administration of Liposomal
Bupivacaine

PRINCIPAL INVESTIGATOR: Chester C. Buckenmaier, MD

CONTRACTING ORGANIZATION: Henry M. Jackson Foundation
Bethesda, MD 20817

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14. ABSTRACT

The project examines the safety aspects of the use of EXPAREL for peripheral nerve blocks. EXPAREL is a novel form of the commonly used bupivacaine, which if injected into the close proximity of nerves, can block the conduction of pain signals, thereby acting as a local anesthetic. Potential adverse events might arise during these procedures, if the drug is accidentally injected into the vasculature. The bupivacaine component of EXPAREL can provoke local anesthetic systemic toxicity (LAST), which in severe cases might cause convulsions and even heart failure. For this reason it is important to determine the tolerable and toxic doses of EXPAREL, and to study the effectiveness of currently recommended rescue protocols for accidental intravascular injection of local anesthetics in animal studies before human clinical trials can be started. The liposomal component of EXPAREL might also have a potential side effect: complement activation related pseudo-anaphylaxis (CARPA), a hypersensitivity reaction that is triggered by some liposomal drugs, and can occur at the first administration, without prior sensitization, and can have a severe outcome. To study the possibility of these reactions we analyze the cardiovascular and immunological reactions following intravenous administration of liposomal bupivacaine in swine, the most sensitive model currently available. Based on the results of the experiments performed during the covered period, intravenously administered Exparel in swine had severe hemodynamic effects at doses as little as 25ul. In rats, asystole is caused by intravenous Exparel at a dose of 40 ml/kg and by bupivacaine at a dose of 13.5 mg/kg, but further experiments to determine maximum tolerable dose and LD50 are required.

15. SUBJECT TERMS

animals, Exparel, liposomal bupivacaine, intravascular, local anesthetic systemic toxicity, complement activation, rats, rodents, swine

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1. **INTRODUCTION:** The project examines the safety aspects of the use of EXPAREL for peripheral nerve blocks. EXPAREL is a novel form of the commonly used bupivacaine, which if injected into the close proximity of nerves, can block the conduction of pain signals, thereby acting as a local anesthetic. If proven to be safe and effective, it could be a viable alternative to continuous infusion of local anesthetics via catheters that can be difficult to place, require expensive pumps, close monitoring, and may be a site for infection. However, potential adverse events might arise during these procedures, if the drug is accidentally injected into the vasculature. The bupivacaine component of EXPAREL can provoke local anesthetic systemic toxicity (LAST), which in severe cases might cause convulsions and even heart failure. It is also necessary to study the effectiveness of currently recommended rescue protocols for accidental intravascular injection of local anesthetics, because certain properties of EXPAREL differ from the conventional solution of bupivacaine. Intravenous lipid emulsion (ILE) has been shown to be effective in bupivacaine toxicity, and the American Heart Association has recommended its use in the Advanced Cardiac Life Support (ACLS) guidelines. However, in EXPAREL the bupivacaine is encapsulated in liposomes, which might decrease the ability of the intravenously administered lipid emulsion to neutralize the systemic toxic effects. Therefore, we conduct experiments to compare the effectiveness of various resuscitative measures using chest compressions alone, and supplemented with lipid emulsion, epinephrine, vasopressin, and their combinations in rats to find the optimal treatment for EXPAREL overdose. The liposomal component of EXPAREL might also have a potential side effect: complement activation related pseudo-anaphylaxis (CARPA), a hypersensitivity reaction that is triggered by some liposomal drugs, and can occur at the first administration, without prior sensitization, and can have a severe outcome. To study the possibility of these reactions we analyze the cardiovascular and immunological reactions following intravenous administration of liposomal bupivacaine in swine, the most sensitive model currently available.
2. **KEYWORDS:** Exparel, liposomal bupivacaine, intravascular, local anesthetic systemic toxicity, complement activation, rats, rodents, swine
3. **ACCOMPLISHMENTS:**
 - **What were the major goals of the project?**
 - Major Task 1 - Protocol preparation and approval process (6 months) – 100% complete
 - Major Task 2 - Rat studies to determine MTD (4 months) – 50% complete
 - Major Task 3 - Rat studies to determine LD50 (4 months) – in progress
 - Major Task 4 - Rat studies to compare effectiveness of ACLS methods for toxicity by EXPAREL (7 months) – not yet started
 - Major Task 5 - Swine experiments to study hypersensitivity to EXPAREL (4 months) – 50% complete
 - Major Task 6 - Publication and final report (5 months) – not yet started
 - **What was accomplished under these goals?**
 - *1) major activities during the period of June 2016 – June 2017: Performance of rat experiments and swine experiments.*
 - *2) specific objectives: Determine Maximum tolerable dose and LD50 of Exparel and Bupivacaine in rats. Determine anaphylactic potential of Exparel in swine*
 - *3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative): In swine, intravenously administered Exparel had severe hemodynamic effects at doses as little as 25ul. In rats, asystoleis caused by intravenous Exparel at a dose of 40 ml/kg and by*

bupivacaine at a dose of 13.5 mg/kg, but further experiments to determine maximum tolerable dose and LD50 are required. The details of the experiments are outlined below.

Rat experiments

Background

The planned research addresses the focus areas of alternatives to current opioid analgesics for severe pain management on the battlefield, remote locations, and in clinical, non-deployed settings. Special emphasis is placed on the safety of an alternative non-opioid analgesic approach utilizing liposomal bupivacaine for peripheral nerve block.

Local anesthetic agents can be used for analgesia by blocking nerve conduction from the site of injury or surgery. Regional anesthesia has played an important role in the care of wounded soldiers in the current conflicts and has become a very important anesthetic technique for managing battlefield casualties. The use of continuous peripheral nerve blocks has become a standard of civilian and military trauma analgesic care. Bupivacaine solutions, a local anesthetic/analgesic widely used in the perioperative and postsurgical settings, have been used for many years by multiple administration routes for the relief of postoperative pain, e.g. via continuous peripheral nerve blocks and direct injection. Continuous infusion of local anesthetics via catheters may extend the duration of the effect, but they can be difficult to place, require expensive pumps, close monitoring, and may be a site for infection.

A recent innovation in the application of local anesthetics is the design of novel drug delivery systems that enable slower, gradual release of the active ingredient, allowing a longer duration of action after a single administration, without the need of continuous infusion. This also leads to a slower uptake into the systemic circulation, eliminating the undesired excessively high peak plasma concentrations and reducing the risk of local and systemic reactions. This would simplify pain management in remote settings and also in the hospital during the postoperative period.

The first FDA approved liposomal local anesthetic formulation, EXPAREL (bupivacaine liposome injectable suspension), is a sterile suspension of multivesicular liposomes created using proprietary DepoFoam formulation technology to release bupivacaine over several days. EXPAREL was designed to provide prolonged analgesia for 72 hours after wound infiltration in patients. A recent study found no local signs of toxicity, including no histological evidence for any increase in local reactions or general exacerbations of bupivacaine toxicity after peripheral nerve block in rabbits and dogs. A single administration of EXPAREL was demonstrated to be safe when tested in comparison with bupivacaine HCl and saline. EXPAREL did not cause overt irritation or local tissue damage even when injected at high dose or concentration around the brachial plexus nerve bundle in these animals.

EXPAREL has recently gained Food and Drug Administration approval exclusively for wound infiltration, however if it proves to be safe and effective, it might become a valuable option for single injection, extended duration peripheral nerve blocks as well. If liposomal bupivacaine proves to be suitable for peripheral nerve block as well, it would provide a valuable alternative to current opioid analgesics for severe pain management by the medic/corpsman on the battlefield and remote

locations as well as for management of acute pain under the care of a clinician in non- deployed settings.

However, before studying the feasibility and effectiveness of the use of liposome encapsulated bupivacaine in human clinical trials the safety of this novel formulation must be comprehensively investigated in animal models. The increased use of local anesthetics drew attention to toxic side effects of these drugs including local tissue toxicity. Inadvertent intravascular injections and the related local anesthetic systemic toxicity (LAST) is one of the most feared complications associated with the use of local anesthetics. Although its incidence is less than 0.2%, LAST is difficult to treat and is potentially fatal.

LAST can impair function of the central nervous system and cause cardiovascular collapse, with potentially lethal consequences. A single study in healthy volunteers with plain bupivacaine has defined the toxic plasma concentration of free bupivacaine to have a range between 0.13 and 0.51 mg/L with a mean value of 0.3 mg/L, but there is no similar published data for EXPAREL. The new formulation probably has different pharmacokinetic properties, and the effects of inadvertent intravascular injection are unknown.

LAST and rescue interventions for its reversal are areas of great interest. Several reports in the recent literature have demonstrated the effectiveness of intravenous lipid emulsion (ILE) in the reversal of cardiovascular and central nervous system symptoms of local anesthetic and other lipophilic drug overdoses. ILE is gaining acceptance and has been included in practice advisories of the American Society of Regional Anesthesia, the Association of Anesthetists of Great Britain, and is also recommended in the Advanced Cardiac Life Support (ACLS) guidelines by the American Heart Association for cardiac arrest secondary to lipophilic beta and calcium channel blockers, when conventional resuscitative therapies have failed.

However, ILE might not be effective in the case of systemic toxicity due to the accidental injection of liposomal bupivacaine into the circulation. The most commonly proposed mechanism of action of ILE is the lipid sink theory. This is based on the lipophilic characteristics of local anesthetics and the partition between the blood plasma and the intravenously injected ILE. According to this theory the lipid sequesters the local anesthetic from the plasma space and prevents its binding to the sites of toxic action in the central nervous system and the heart. However, liposomal formulations of local anesthetics (EXPAREL in particular) are already encapsulated in liposomes and the applicability of lipid rescue in the case of their inadvertent intravascular injection is questionable.

Methods

In the first phase of the project a dose response study was performed with continuous infusions of (a) EXPAREL and (b) plain bupivacaine correlating them with cardiovascular symptoms. Our hypothesis is that the maximum tolerated dose (MTD) of EXPAREL is higher than the MTD of plain bupivacaine. Maximum tolerated dose is defined as the highest dose level at which $\leq 33\%$ of subjects experience dose-limiting toxicities. We are going to define both MTDA and MTDX. For MTDA the dose-limiting toxicity will be signs of arrhythmia and for MTDX the dose-limiting toxicity will be asystole.

Rats will be anesthetized in a bell jar with isoflurane to allow tracheal intubation. All animals will then be placed on a heated stand under a warming lamp and mechanically ventilated with 1-2% isoflurane in 100% oxygen, using a rodent ventilator (RoVent Jr., Kent Scientific, Torrington, CT) to deliver a tidal volume of 2.5 ml at a starting rate of 65-70 breaths/min.

A total of 26 rats (13 in the EXPAREL group and 13 in the plain bupivacaine group) will be anesthetized with isoflurane, intubated, and ventilated with 1-2% isoflurane in 100% oxygen. Catheters are inserted into the left femoral vein (22GA angicath, BD, Franklin Lakes, NJ), the left femoral artery (22GA angiocath, BD, Franklin Lakes, NJ), for hemodynamic monitoring and blood sampling. Arterial blood pressure and ECG was continuously monitored and recorded by a PowerLab data acquisition system and Chart 5 software (AdInstruments, Colorado Springs, CO) After a 10 minutes stabilization period a continuous intravenous infusion of the test substances was administered at 10 mg/kg/min. The primary endpoints were total dose and time to arrhythmia (MTDA), total dose and time to 50% of baseline of mean arterial pressure, and total dose and time to asystole (MTDX). Blood samples of 2 ml (will be collected at the time of asystole for plasma drug concentration analysis in the future.

Results

During the period covered by the report the methodology has been established and experiments to determined MTDA and MTDX has been started.

The financial account for the grant has been established at USU and funds have been transferred from HJF to USU for animal orders and related charges.

The experiments with rats to determine maximum tolerable dose have been started. After the first 4 experiments, it was determined that the respiration of the animals needs to be controlled with a rodent ventilator. The equipment has been selected, acquired and set up.

After finalizing the methodology for the experiments, 4 additional experiments have been completed with controlled ventilation and the project is in progress.

Rat 01 (680g) has been anesthetized and instrumented. While hemodynamic parameters were continuously monitored, Exparel was infused into the femoral vein at a rate of 1 ml/min. First signs of arrhythmia were noted 77 seconds later. The dose injected by the time of arrhythmia was 25mg/kg. Asystole was noted 123 seconds after beginning of the infusion. The total dose injected by the time of asystole was 40 mg/kg.

Rat 02 (767g) has been anesthetized and instrumented. While hemodynamic parameters were continuously monitored, Bupivacaine 0.5% was infused into the femoral vein at a rate of 0.2 ml/min (1.3mg/kg/min). First signs of arrhythmia were noted 83 seconds later (1.8mg/kg infused), which proved to be transient. Arrhythmia developed again starting at 293 seconds (6.35 mg/kg infused) and became gradually more severe. Asystole was noted at 3702 seconds. Total dose of Bupivacaine infused by the time of asystole was 80 mg/kg.

The slow infusion in Rat 02 resulted in a much higher than expected dose required for the development of arrhythmia and asystole. Based on previous study and the literature the dose of bupivacaine used in a rat model of local anesthetic toxicity is 10-20 mg/kg. In order to make our model comparable to other studies we increased the rate of infusion of the test substances to 10 mg/kg/min.

Rat 03 (960g) has been anesthetized and instrumented. While hemodynamic parameters were continuously monitored, Exparel was infused into the femoral vein at a rate of 10 mg/kg/min (0.72 ml/min). First signs of arrhythmia were noted at 64 seconds (10.7 mg/kg infused), which became gradually more severe. Asystole was noted at 240 seconds. Total dose of Exparel infused by the time of asystole was 40 mg/kg.

Rat 04 (771g) has been anesthetized and instrumented. While hemodynamic parameters were continuously monitored, Exparel was infused into the femoral vein at a rate of 10 mg/kg/min (0.58 ml/min). First signs of arrhythmia were noted at 40 seconds (6.7 mg/kg infused), which became gradually more severe. Blood pressure gradually decreased and at 245 seconds (41 mg/kg infused) the pulse was lost and the rhythm was considered pulseless electrical activity. Asystole was noted at 300 seconds. Total dose of Exparel infused by the time of asystole was 50 mg/kg.

Rat 05 (880g) has been anesthetized and instrumented. While hemodynamic parameters were continuously monitored, 0.5% Bupivacaine was infused into the femoral vein at a rate of 10 mg/kg/min (1.76 ml/min). First signs of arrhythmia were noted at 20 seconds (3.3 mg/kg infused), which became gradually more severe. Asystole was noted at 81 seconds. Total dose of Bupivacaine infused by the time of asystole was 13.5 mg/kg.

Conclusions

Mechanical ventilation is required in our rat experimental model of local anesthetic systemic toxicity. The infusion rate of 1 mg/kg/min 0.5% bupivacaine is too slow to get results that are consistent with previous studies and the literature. Increasing the rate of infusion to 10 mg/kg/min provided adequate results for both Exparel and Bupivacaine.

Our preliminary results show that the MTDA and MTDX for Exparel is at least twice as much as for bupivacaine.

Further experiments are needed to verify the results and establish LD50.

Swine experiments

Background

An additional safety concern during inadvertent intravascular injection of liposome-encapsulated bupivacaine arises from the liposome component of the formulation. A large variety of chemical substances as well as medicinal products have been suspected or proven to provoke adverse immunological reactions. One of the most common side effects of drugs is hypersensitivity reaction that often prevents or limits their use. In the past decade several drugs and chemicals were shown to have a potential to trigger complement activation related pseudo-anaphylaxis (CARPA).

(15) These also include but are not limited to liposomal formulations such as Doxil and Ambisome. (16)

The monitoring of CARPA became an important aspect in the development of these pharmaceuticals. Underlying the importance of this new type of hypersensitivity, in vitro and in vivo testing of complement activation became a recommended toxicology test by the US Food and Drug Administration. (17)

Unlike IgE-mediated allergy, these reactions arise without prior sensitization and are mediated by the complement system. The outcomes include activation of mast cells, polymorphonuclear cells and platelets, the release of vasoactive mediators, such as thromboxane and histamine, with severe

cardiovascular and other effects, with potentially lethal consequences. (18) To study the possibility of these reactions we intend to analyze the hemodynamic and immunological reactions following intravenous administration of liposomal bupivacaine in swine.

Methods

Five Yorkshire swine (20 - 25 kg) were sedated with ketamine (15-25 mg/kg IM, 18-19 gauge needle, dorsolateral neck muscles), and anesthesia was induced with cone-mask inhalation of 3-4% isoflurane. After tracheal intubation (6.5-7.0 mm endotracheal tube), ventilation was controlled (100 ml/kg/min, Narkomed 2B; North American Drager, Telford, PA), and anesthesia was maintained with 1-4% inhaled isoflurane as needed. Waste gas from the anesthesia machine was scavenged via carbon filter. A catheter (18 gauge) was placed in the right ear vein for IV maintenance fluids (0.9% NaCl at 1-2 ml/kg/hr) and injection of liposomal bupivacaine (Exparel, Pacira Pharmaceuticals, Parsippany, NJ). An 18 gauge catheter was inserted percutaneously into the superficial femoral artery to provide access for serial blood sampling and systemic arterial blood pressure (SAP) and heart rate (HR) monitoring. A 9 Fr Cordis percutaneous introducer was placed into the right external jugular vein, and a Swan-Ganz catheter was floated through the right atrium and right ventricle to the pulmonary artery for pulmonary arterial pressure (PAP) measurements. Continual respiratory rate (RR), end-tidal carbon dioxide (ETCO₂), EKG, and rectal temperature was monitored (M1026A Gas Analyzer and Model 68 clinical monitor; Hewlett-Packard, Andover, MD). Normal body temperature (37-38 degree C) was maintained by an external warming device.

Upon completion of instrumentation and a 15- minute stabilization period, baseline blood samples were taken for biochemical analyses. All blood samples drawn during the experiment were 10 ml into tubes containing EDTA, centrifuged, plasma separated and frozen for later immunologic and complement assays.

After the baseline blood draw, bolus doses of Exparel were injected via the femoral arterial catheter according to the injection schedule described below. Hemodynamic parameters were recorded continuously. Blood samples of 10 ml were collected at 3, 6, 9, and 12 minutes after each injection. There was at least 20 minutes of stabilization period between boluses to allow the return of hemodynamic parameters to baseline.

Pig 01 (21.5 kg): 1 ml Exparel, 1 ml Exparel, 1 ml Exparel, 0.5 mg/kg Zymosan

Pig 02 (21.8 kg): 1 ml Exparel, 1 ml Exparel, 1 ml Exparel, 0.5 mg/kg Zymosan

Pig 03 (20.4 kg): 100 ul Exparel, 50 ul Exparel, 25 ul Exparel, 12.5 ul Exparel, 5 ul Exparel, 200 ul Exparel, 400 ul Exparel, 1.5 ml Exparel

Pig 04 (22.7 kg): 5 ul Exparel, 12.5 ul Exparel, 25 ul Exparel, 50 u Exparel, 100 ul Exparel, 200 ul Exparel

Pig 05 (25 kg): 5 mg/kg indomethacin in 8.5 ml ethanol, 50 ul Exparel, 1 ml Exparel, 1 ml Exparel, 1 ml Exparel, 1 ml Exparel, 10 ml Exparel

At the end of the experiments the animals were euthanized with Euthasol provided by LAM. The maximum time that any pig was maintained from initiation of anesthesia until euthanasia was 3 hours.

Results

Changes in the hemodynamic parameters are summarized in Table 1.

animal ID	treatment	change in PAP (mmHg)	change in SAP (mmHg)	change in ETCO2 (mmHg)	comments
01	1ml Exparel	17 -> 51	65 -> 107	41 -> 53	dyspnea
	1ml Exparel	17 -> 40	63 -> 85	47 -> 51	
	1ml Exparel	19 -> 43	69 -> 87	49 -> 53	
	0.5mg/kg Zymosan	23 -> 49	77 -> 113	60 -> 20	apnea
02	1ml Exparel	17 -> 47	64 -> 92	54 -> 59	
	1ml Exparel	18 -> 42	65 -> 84	56 -> 58	
	1ml Exparel	18 -> 43	68 -> 51	56 -> 51	
	0.5mg/kg Zymosan	18 -> 43	68 -> 82 -> 15	56 -> 0	apnea/circulatory collapse
3	100ul Exparel	17 -> 33	80 -> 85	47 -> 52	
	50ul Exparel	15 -> 24	80 -> 84	46 -> 50	
	25ul Exparel	15 -> 22	79 -> 83	45 -> 46	
	12.5ul Exparel	16 -> 20	80 -> 82	43 -> 45	
	5ul Exparel	16 -> 18	82 -> 84	44 -> 43	
	200ul Exparel	15 -> 42	80 -> 39	44 -> 48	circulatory collapse
	400ul Exparel	15 -> 42	71 -> 36	42 -> 31	circulatory collapse
	1.5ml Exparel	15 -> 47	69 -> 30	36 -> 16	apnea/circulatory collapse
4	5ul Exparel	11 -> 15	75 -> 76	52 -> 51	
	12.5ul Exparel	11 -> 26	73 -> 73	50 -> 51	
	25ul Exparel	11 -> 31	76 -> 83	50 -> 41	
	50ul Exparel	11 -> 37	77 -> 25	48 -> 41	apnea/circulatory collapse
	100ul Exparel	10 -> 34	71 -> 15	53 -> 22	circulatory collapse
	200ul Exparel	11 -> 30	54 -> 12	52 -> 17	apnea/circulatory collapse
5	indomethacin	14 -> 28	68 -> 107	53 -> 60	
	50ul Exparel	20 -> 18	89 -> 89	56 -> 55	
	1ml Exparel	18 -> 18	90 -> 88	55 -> 38	gradual increase in respiratory rate, decrease in ETCO2
	1ml Exparel	19 -> 19	94 -> 83	37 -> 45	
	1ml Exparel	17 -> 18	78 -> 92	41 -> 43	
	1ml Exparel	18 -> 18	76 -> 81	42 -> 43	
	10ml Exparel	18 -> 39	75 -> 66 -> 92	43 -> 39	

Table 1. Hemodynamic changes following injection of study drugs.

In pig 01, administration of 1ml Exparel via the femoral artery caused an increase in PAP, SAP and ETCO2, with severe dyspnea. To confirm the results the bolus was repeated, and there was an increase, although slightly smaller, observed in PAP, SAP and ETCO2. To see if the reaction can be exhausted we injected a third dose of 1ml Exparel, which caused the same reaction. Zymosan, a known complement activator used as a positive control caused a similar reaction, only the respiratory distress was more severe.

The experiment was repeated in pig 02 to confirm the results and the hemodynamic changes following the injections was essentially identical. In consensus with previous studies investigating complement activation related pseudoanaphylaxis, we found that the benchmark parameter would be the change in pulmonary arterial pressure.

In pig 03 we injected decreasing doses of Exparel to find the lowest dose that still provokes the reaction. 25 and 50ul Exparel caused an approximately 50% increase in the pulmonary arterial pressure. 12.5ul Exparel caused a 25% increase.

To mitigate the effect of potential tachyphylaxis, in pig 04 we reversed the order of injections and administered escalating doses of Exparel. 12.5 ul Exparel caused a more than 100% increase in PAP. (Figure 1) 50ul Exparel caused a transient circulatory collapse with the SAP rapidly falling to 25mmHg followed by a quick recovery.

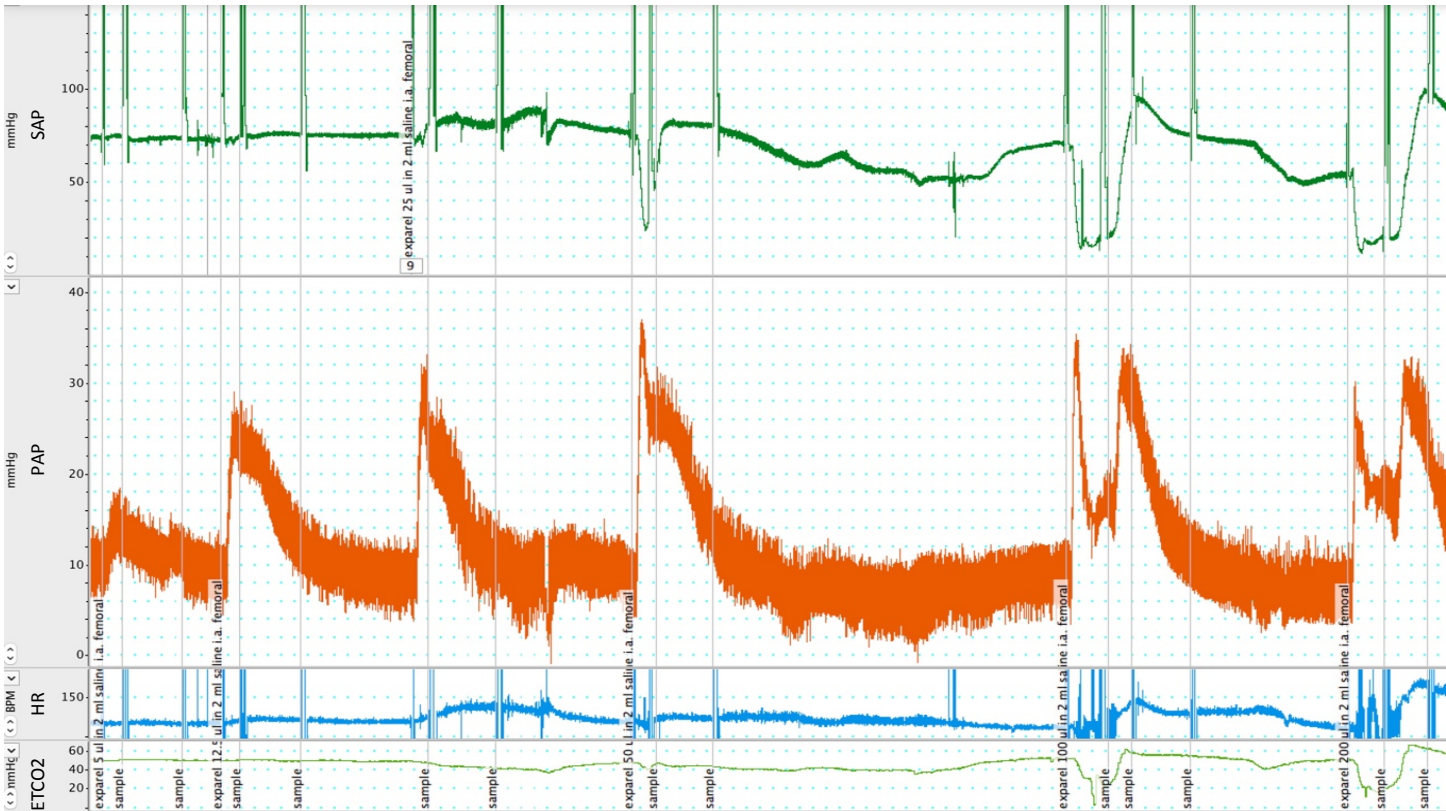


Figure 1. Systemic arterial pressure (SAP), pulmonary arterial pressure (PAP), heart rate (HR), and end tidal CO2 (ETCO2) during escalating doses of Exparel.

To investigate the role of prostaglandins in the reaction we treated pig 05 with 5 mg/kg indomethacin before injecting escalating doses of Exparel. Based on previous studies and the literature we hypothesize that thromboxane might play a central role in the mechanism of the hemodynamic reactions. The indomethacin treatment seemed to prevent the changes provoked by administration of Exparel in pig 05. (Figure 2) The PAP, SAP, ETCO₂ all remained essentially unchanged compared to baseline after injections of 1ml Exparel. To find out if the desensitization has a limit we injected 10 ml Exparel at the end of the experiment, which resulted in an increase in PAP and mild changes in SAP, showing that the protection by inhibiting the cyclooxygenase cascade has limits.

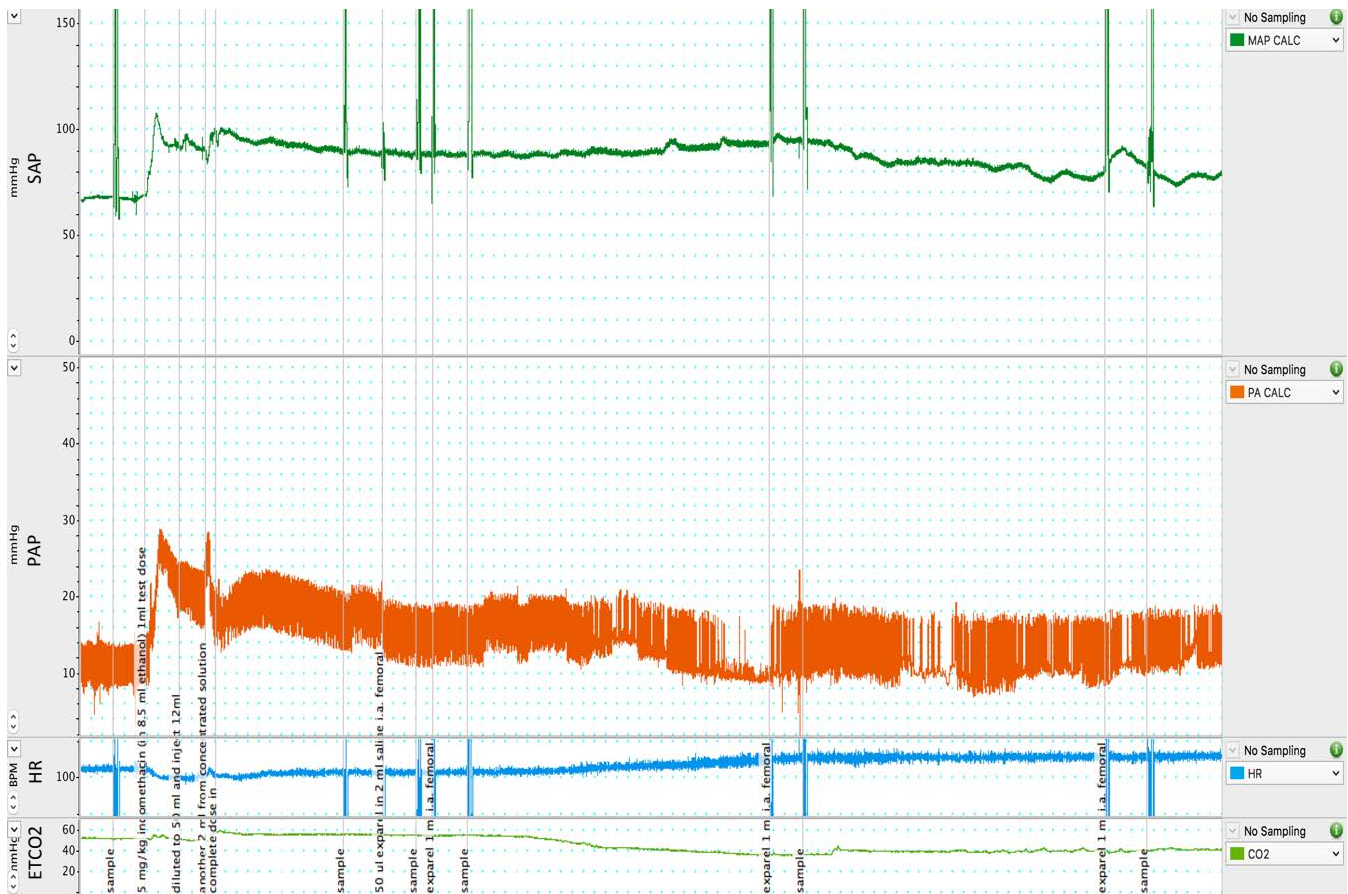


Figure 2. Systemic arterial pressure (SAP), pulmonary arterial pressure (PAP), heart rate (HR), and end tidal CO₂ (ETCO₂) during escalating doses of Exparel after pretreatment with indomethacin.

Conclusions

Injection of Exparel into the femoral artery of pigs in doses of at least 25ul provokes hemodynamic changes, characterized by significant dose dependent increase in pulmonary arterial pressure, increase or decrease in systemic arterial pressure, increase in end tidal CO₂, respiratory distress. Inhibition of the cyclooxygenase cascade by injections of 5 mg/kg indomethacin prevented the hemodynamic reactions to intraarterial injection of 1 ml Exparel, but subsequent injection of 10 ml Exparel caused an approximately 100% increase in pulmonary arterial pressure.

- - 4) other achievements: nothing to report
- **What opportunities for training and professional development has the project provided?**
 - *Nothing to report*
- **How were the results disseminated to communities of interest?**
 - *Nothing to report*
- **What do you plan to do during the next reporting period to accomplish the goals?**
 - *Complete the rat experiments to determine LD50. Complete rat experiment comparing resuscitative methods.*

4. IMPACT

- **What was the impact on the development of the principal discipline(s) of the project?**
 - *Our findings from the swine experiments raise a significant and novel characteristic of Exparel, which might have important implications for clinical use and guidelines. The hemodynamic reactions observed after intravenous administration of Exparel in swine may also occur in humans, in which case preventive measures must be taken every time Exparel is used. The results from the swine experiments might have an impact on the FDA approval status of Exparel and its use in clinical practice.*
- **What was the impact on other disciplines?**
 - *Nothing to report*
- **What was the impact on technology transfer?**
 - *Nothing to report.*
- **What was the impact on society beyond science and technology?**
 - *Nothing to report*

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**
 - *Nothing to report*
- **Actual or anticipated problems or delays and actions or plans to resolve them**
 - *There has been a delay with the availability of funds. Due to this complication, we began the project in Spring of 2015 and all projected milestones will be delayed accordingly.*
- **Changes that had a significant impact on expenditures**
 - *Nothing to report*
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
 - *Nothing to report*
- **Significant changes in use or care of human subjects**
 - *Nothing to report*
- **Significant changes in use or care of vertebrate animals.**
 - *Nothing to report*
- **Significant changes in use of biohazards and/or select agents**
 - *Nothing to report*

6. PRODUCTS: *Nothing to report*

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

○ **What individuals have worked on the project?**

Name:	Chester Buckenmaier
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	Dr. Buckenmaier provided scientific and administrative oversight
Funding Support:	USUHS
Name:	Peter Bedocs
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	Dr. Bedocs designed the protocol, coordinated submission and review correspondence, trained in methodology, designed and performed experiments and performed laboratory setup
Funding Support:	W81XWH-15-1-0130
Name:	Kelly Kiser
Project Role:	Program Manager
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	Ms. Kiser provided administrative support
Funding Support:	W81XWH-15-1-0130

○ **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

▪ *Nothing to report*

○ **What other organizations were involved as partners?**

▪ *Nothing to report*

8. SPECIAL REPORTING REQUIREMENTS

▪ *Nothing to report*

9. APPENDICES: *Nothing to report*